LC50 Aquatic Toxicity Test Results for Ammonium Perchlorate-
A Two-Species Chronic Definitive Bioassay

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BES Sample #16277

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1. INTRODUCTION

The Federal Water Pollution Control Act Amendments of 1972 (PL 92-500), the Clean Water Act (CWA) of 1977 (PL 95-217), and the Water Quality Act of 1987 (PL 100-4) explicitly state that it is the national policy that the discharge of toxic substances in toxic amounts be prohibited. Toxicity to aquatic life is one of the criteria used to gauge the hazardous potential of a discharged waste. The type of toxicity test and particular species used for testing of effluents is dictated under the framework of the National Pollutant Discharge Elimination System and falls under the jurisdiction of the local Regional Water Quality Control Board.

Block Environmental Services (BES) has conducted a chronic LC50 definitive bioassay on ammonium perchlorate, using two different species, for the Santa Clara Valley Water District through Clayton Group Services. The toxicity testing organisms of interest are the daphnid (*Ceriodaphnia dubia*) and fathead minnow (*Pimephales promelas*). Each test was performed under definitive conditions for both the range finding and definitive determinations of toxicity. As a QA/QC measure, a standard reference toxicant test was run concurrently for each of the two species. This report describes the procedures used and the results obtained for the toxicity tests initiated on July 7, 1998.

BES is an Environmental Laboratory Accreditation Program certified laboratory (#1812).

2. MATERIALS AND METHODS

2.1 SAMPLE COLLECTION AND HANDLING

2.1.1 Sample Collection - Water for the laboratory study was prepared from a super stock solution made from reagent grade ammonium perchlorate (purchased from Alfa AESAR; Lot #C16I20). The super stock solution was prepared to a nominal concentration of 20,000 mg ClO_4^-/L. Samples were prepared each day from the super stock solution and diluted to the appropriate concentration for testing. In addition, 250 ml samples were collected of the ammonium perchlorate super stock solution and the control water for each test species prior to the definitive tests for laboratory analysis of perchlorate by the Santa Clara Valley Water District.

2.1.2 Reference Toxicant - Sodium Chloride (NaCl) was used as the reference toxicant for the daphnid (*C. dubia*). A stock solution containing 16g/L NaCl was used for daily preparation of the treatment levels for these organisms. The *C. dubia* treatment levels were 4, 2, 1, 0.5 and 0.25 g/L NaCl in USEPA Moderately Hard Water. Potassium Chromate (K_2CrO_4) was used as the reference toxicant for the fathead minnow (*P. promelas*). A stock solution containing 20 g/L Cr^{+6} was used for daily preparation of the treatment levels of 67.7, 33.8, 16.9, 8.5, and 4.2 mg/L Cr^{+6} in USEPA Hard Water. The toxicity endpoints from the reference toxicant tests of each test method are subsequently plotted on a running control chart from the last 20 tests. The mean values as well as the upper and lower control limits (± 2 standard deviations) are recalculated with each successive test result. The outliers, which are values falling outside the upper and lower control limits, and trends of increasing or decreasing sensitivity, are readily identified.

2.2 TOXICITY TEST PROCEDURES

2.2.1 Test Procedures - A detailed procedure for each test is outlined in laboratory standard operating procedures (SOPs) kept at the BES laboratory and they are based upon the following reference:

- Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms (EPA/600/4-91/002).

2.2.2 Data Analysis - All toxicity testing results were analyzed using the EcoAnalysis, Inc. software program TOXIS (Version 2.5). This program determines if there is a statistically significant reduction in response at the $p = 0.05$ level and utilizes the flowchart for statistical analysis outlined in EPA/600/4-91/002. The testing compared the sample responses with the laboratory control water. The initial rangefinding tests were performed with exposures separated by a factor of ten. The definitive tests subsequently used the results from the rangefinding tests to obtain a more accurate determination of the ammonium perchlorate toxicity to each organism. The parameters of interest are the (50%) Lethal Concentration, the No Observed Effect Concentration (NOEC), the Lowest Observed Effect Concentration (LOEC), and the (25 and 50%) Inhibition Concentrations (IC). The LC/IC will show the point estimate of the sample concentration that causes a given percent reduction. Statistical calculations are based upon the nominal concentration values. The measured statistical enpoints were determined by adjusting the nominal results by the analytical perchlorate values.

3. RESULTS

3.1 ROUTINE REPORTING

3.1.1 Sample Identification - Ammonium Perchlorate (expressed as mg ClO_4^-/L)

3.1.2 Test Species -

- daphnid (*Ceriodaphnia dubia*) at <24 hours old
- fathead minnow (*Pimephales promelas*) at <24 hours old.

3.1.3 Summary Of Perchlorate Analysis - These values represent the analysis of the perchlorate levels of the samples as received at the Santa Clara Valley Water District.

Sample ID	Perchlorate (mg ClO_4^-/L)
C. dubia control	0.045
P. promelas control	0.005
20,000 mg ClO_4^-/L super stock	19,200

3.1.3 Rangefinder Test Initiation Dates -

- *C. dubia* = 7/7/98
- *P. promelas* = 7/7/98

3.1.4 Definitive Test Initiation Dates -

- *C. dubia* = 7/14/98
- *P. promelas* = 7/14/98

3.1.5 *C. dubia* End Point Values -

Rangefinder Raw Data

Sample Conc. (mg ClO_4^-/L)	6 day Percent	6 day Neonate Reproduction	
Nominal	Survival	# per female	S.D.
Control	100	24.20	2.168
0.15	100	21.80	5.675
1.5	100	23.80	3.701
15	80	16.20	8.044
150	0	0	0
1500	0	0	0

Definitive Raw Data

Sample Conc. (mg ClO_4^-/L)		6 day Percent	6 day Neonate Reproduction	
Nominal	Measured	Survival	# per female	S.D.
Control	0.045	90	21.70	7.617
1	1.005	100	24.00	6.000
10	9.645	100	22.00	4.447
25	24.045	100	8.80	4.686
100	96.045	40	0	0
200	192.045	0	0	0

Definitive Statistical Analysis (mg ClO_4^-/L)

Survival End Point	LC50	NOEC	LOEC
Nominal	80.981	25	100
Measured	77.787	24.045	96.045

Reproduction End Point	IC50	IC 25	NOEC	LOEC
Nominal	22.017	15.526	10	25
Measured	21.181	14.949	9.645	24.045

3.1.5.1 Testing Notes

The laboratory control water passed all three acceptability criteria for both tests: survival (≥ 80%), number of broods (≥ 60% of the surviving adults must have had at least three broods), and average number of neonates (≥ 15 neonates/adult).

The concurrent reference toxicant tests generated survival and reproduction endpoints that were within the control chart limitations. This *C. dubia* response indicates that the values elicited for the ammonium perchlorate LC50 determination are valid.

3.1.6 *P. promelas* End Point Values -

Rangefinder Raw Data

Sample Conc. (mg ClO_4^-/L)	7 day Percent Survival		7 day Dry Weight	
Nominal	Average (%)	S.D.	Average (mg)	S.D.
Control	100	0	0.70	0.127
0.15	95	7.1	0.59	0.042
1.5	100	0	0.72	0.021
15	100	0	0.68	0.042
150	90	0	0.42	0.042
1500	0	0	0	0

Definitive Raw Data

Sample Conc. (mg ClO_4^-/L)		7 day Percent Survival		7 day Dry Weight	
Nominal	Measured	Average (%)	S.D.	Average (mg)	S.D.
Control	0.005	100	0	0.69	0.076
10	9.605	100	0	0.68	0.056
100	96.005	100	0	0.57	0.036
250	240.005	50	20	0.13	0.055
1000	960.005	0	0	0	0
2000	1920.005	0	0	0	0

Definitive Statistical Analysis (mg ClO_4^-/L)

Survival End Point	LC50	NOEC	LOEC
Nominal	281.171	100	250
Measured	269.929	96.005	240.005

Growth End Point	IC50	IC 25	NOEC	LOEC
Nominal	177.557	119.176	10	100
Measured	170.460	114.414	9.605	96.005

3.1.6.1 Testing Notes

The laboratory control water passed both the survival (≥ 80%) and growth (≥ 0.25 mg/surviving adult) acceptability criteria for both tests.

The concurrent reference toxicant tests generated survival and growth endpoints that were within the control chart limitations. This *P. promelas* response indicates that the values elicited for the ammonium perchlorate LC50 determination are valid.